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INCREASED CD4+CD25HIGH+ REGULATORY T-CELL ARE ASSOCIATED WITH DISEASE RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION (SCT) FOR CHRONIC MYELOID LEUKAEMIA (CML)

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The success of SCT after CML largely relies on the graft versus leukaemia (GvL) effect exert by donor T-cells. CD4+CD25+ regulatory T-cell (Tregs) play a crucial role in the maintenance of peripheral tolerance and have been tested in animal models to successfully prevent GVHD. The role of Tregs in clinical transplantation remains unclear, insofar as the few studies published to date have reported controversial results regarding GvHD. Although there is emerging evidence that Tregs are associated with a poor outcome in cancer patients, none of these studies has investigated the role of Tregs in leukaemia relapse post-SCT.

To address this question we quantified CD4CD25high regulatory T-cells in post-SCT patients and correlated their levels with clinical outcome.

We performed a cross-sectional study at a single institution. We enumerated and characterised peripheral blood CD4+CD25high+ Tregs in 76 patients after allogeneic SCT for CML by FACS analysis. As control we analysed 21 samples from healthy volunteers and 20 samples from newly diagnosed CML patients. BCR-ABL/ABL ratio was determined in every sample by real-time PCR. Patients were considered in remission if the ratio was less than 0.02% and in relapse if higher. All quoted p-values are two-sided with $P<0.05$ considered statistically significant.

Patients after SCT had higher levels of Treg than normal donors (median 1.5% vs 0.87, $p<0.01$) and untreated CML (median 1.5% vs 0.27, $p<0.0001$). In the multiple regression analysis only the time post SCT (before or after 18 months) and disease status (molecular remission versus relapse) were predictive for increased Tregs (Coef -2.994, $p=0.004$ and Coef -2.395, $p=0.020$ respectively). No association with Treg levels and GvHD was found. The logistic regression analysis performed in 43 patients that had not received DLI post SCT confirmed that increased Tregs, both as percentage or absolute numbers, were the only predictive variable for relapse (exp 1.44, $p=0.011$).

A substantial expansion of Tregs occurs early after allogeneic SCT and the presence of high numbers of Tregs 18 months after transplant is predictive of leukaemic relapse. Although the increment might initially have an advantageous effect on graft rejection, our data suggest that Tregs exert an inhibitory effect on GvL.

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PRESERVED ANTI-VIRAL RESPONSES AND IMPROVED SURVIVAL IN STEROID REFRACTORY GVHD USING A COMBINATION OF DACLIZUMAB AND INFLIXIMAB

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Steroid refractory acute graft versus host disease (SRGvHD) is a life threatening complication of SCT with poor prognosis due to infection related mortality. We investigated the combined use of Dacluzimab, a humanised monoclonal antibody (Mab) targeting IL-2 and Infliximab, a chimeric human/mouse anti-TNF a, in order to selectively delete alloreactive cells and target 2 different points in the cytokine cascade responsible for aGvHD.

Fifteen consecutive children (median age 4.5 years) with SRGvHD (defined as aGvHD that failed to improve after 1 week of at least 2mg/kg/day of Methylprednisolone) were treated. Donors were MSD or MFD (*n=3*), MUD (*n=9*) and mismatched UD (*n=3*). All 15 patients had involvement of the skin, 14 of the lower gut and 5 of the liver. All patients had grade 3 (*n=5*) or 4 (*n=10*) GVHD. Patients were treated with a combination of Daclizumab (1mg/kg, days 1,4,8,15,22) and Infliximab (10mg/kg, days 1,8,15,22), with rapid reduction of steroid dosage to ≤ 1mg/kg. Median time of starting the Mabs was day 17 from the onset of GvHD. All children received anti-fungal prophylaxis and prospective viral monitoring.

12/15 patients responded (7 CR, 5 PR), with a median response time of 13 days. Two patients developed recurrent GvHD and received a second course of Mabs. There were 10 episodes of viral reactivations (CMV 4, adeno 3, EBV 3) and 3 patients developed probable fungal infections. Impressively, however, there were no infection related deaths. T-cell responses to CMV after mAb infusion were assessed in 5 patients using the IFN-g ELISPOT assay. As shown in the table below, 4 patients showed a significant response to CMV (defined as $>$ 40 SFC per 2×10^5 PBMC) in the first 3 months after treatment and at least 1 patient (UPN2) was able to mount a *de novo* response to CMV after mAb therapy.

At a median follow-up of 30 months, 10/15 (66%) children are alive. 3 primary non-responders died of progressive GvHD, as did 1 patient who achieved a PR but subsequently developed bronchiolitis obliterans and 1 patient who died of Pseudomonas sepsis. 5/10 evaluable patients developed chronic GVHD (3 limited, 2 extensive), which has resolved in 2 cases. This data suggests that combination treatment with Daclizumab and Infliximab is a highly effective therapeutic option for patients with SRGvHD and is associated with preservation of anti-viral responses and a low infection related mortality compared to standard therapies.

CMV-specific T-cell responses post- mAbs (IFN-gamma SFC/2 × 10e5 PBMC)

	Pre-mAbs	1 month	3 months
UPN 1	**NE**	**0**	**41**
UPN 2	**0**	**2**	**98**
UPN 13	**81**	**138**	**23**
UPN 14	**NE**	**73**	**148**
UPN 15	**11**	**NE**	**0**
Normal donor	**68**		

NE= not evaluated

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HISTONE DEACETYLASE INHIBITORS INDUCE INDOLEAMINE 2, 3-DIOXYGENASE AND MODULATE DENDRITIC CELL FUNCTIONS

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Recent studies have demonstrated that suberoylanilide hydroxamic acid (SAHA), a histone deacetylase (HDAC) inhibitor, reduces experimental acute graft-versus-host disease (GVHD). We investigated the cellular-molecular mechanisms of immuno-modulation by two HDAC inhibitors, SAHA and ITF 2357. Stimulation of bone marrow derived dendritic cells (DCs) with various TLR ligands (lipopolysaccharide (LPS), PGN, CpG) after pretreatment with either SAHA caused a significant reduction in the secretion of TNF-α, IL-12p70 and IL-6 compared to the untreated controls ($P< 0.01$). Similar effects were seen using *human* peripheral blood mononuclear cell derived DCs. Pre-treatment of DCs also significantly reduced their *in vitro* and *in vivo* stimulation of allogeneic T cells as measured by proliferation and IFN-γ production ($P<0.05$), which was not reversed by anti-IL-10 or anti-TGFβ. No significant difference was observed in the viability of pretreated and control DCs. Pretreatment significantly suppressed the expression of CD40 and CD80. When mixed with normal DCs at 1:1 ratio, SAHA treated DCs suppressed allogeneic T cell responses in a contact dependent manner. When DCs from B6 MHC Class II deficient ($H\text{-}2^b$) were treated with SAHA and co-cultured with wild type B6 DCs along with purified allogeneic BALB/c ($H\text{-}2^d$) $CD4^+$ T cells in an MLR, the allo-$CD4^+$ T cells proliferated demonstrating the regulation to be dependent on contact between SAHA treated DCs and T cells. To determine the molecular mechanism, we analyzed for the expression of indoleamine 2, 3-dioxygenase (IDO) and found that both SAHA and ITF 2357 increased IDO at the mRNA and protein levels. A functional role for IDO was confirmed by the blockade of its induction with specific small interfering RNA (siRNA) in SAHA mediated suppression if TNF- α. To address the *in vivo* relevance of this suppression, we utilized the [BALB/c →B6] model of acute GVHD. B6 animals received 11Gy on day -1 and injected with of 5 million B6 SAHA treated or control DCs